REMARKS

This document is submitted in response to the Office Action mailed June 30, 2005 ("Office Action"). Applicants have amended claims 12, 13, 21, 24, and 40, and have added new claim 75 has been added. Applicants have cancelled claims 1, 11, 17, 37, 41, and 46. Claims 22, 23, 38, 47-50, 52-54, and 56-74 were previously withdrawn. Support for the amendments and new claims can be found in the original claims and in the specification as will be pointed out in the remarks below.

Upon entry of the proposed amendments, claims 5, 12, 13, 21, 24, 40, 55, and 75 will be under examination. Reconsideration of the application is respectfully requested in view of the remarks below.

Rejections under 35 U.S.C. § 112, First Paragraph (Enablement)

Claims 1, 5, 17, 21, 24, 40, and 45 were rejected as allegedly lacking enablement.

The Office Action ("Office Action") takes issue with the breadth of these claims, stating:

> These claims are so broad as to encompass any *E. coli* having an inactivating *gnL* mutation which are transformed with a nucleic acid encoding any biosynthetic enzyme for the production of any isoprenoid, any polyketide or any polyhydroxyalkanoate and the use of such cells for the production of any isoprenoid, any polyketide or any polyhydroxyalkanoate. The scope of each of these claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of genes necessary for the construction of the host cells broadly encompassed by the claims nor for the use of all such cells for the production of any isoprenoid, any polyketide or any polyhydroxyalkanoate." (Office Action, page 5, lines 18-23; and page 6, lines 1-6).

As noted above, claims 1 and 17 were cancelled. As to the remaining claims, Applicants turn first to amended independent claims 24 and 40, as well as their dependent claims.

Independent claim 24, as amended covers a kit including, *inter alia*, a coding sequence for specific isoprenoid biosynthetic enzymes, i.e., isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, 1-deoxyxylulose 5-phosphate synthase, a phytoene synthase, or a phytoene desaturase. The specification teaches that these enzymes are useful for

isoprenoid biosynthesis, e.g, at page 5, lines 1-5. Claim 45 has been amended to depend from claim 24.

Similarly, independent claim 40, covering an *E. coli* bacterial host cell, has been amended to recite a nucleic acid encoding a specific biosynthetic enzyme, i.e., isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, 1-deoxyxylulose 5-phosphate synthase, phosphoenolpyruvate synthase, 3-ketoacyl reductase, poly-3 hydroxyalkanoate polymerase, a phytoene synthase, a phytoene saturase, lycopene cyclase, or farnesyl diphosphate synthase. The specification teaches that these enzymes are useful for biosynthesis of an isoprenoid or a polyhydroxyalkonate, e.g., page 5, lines 15-22.

Claim 5 has been amended to depend from claim 12, which is currently amended to be an independent claim. Amended claim 12 covers an *E. coli* host cell that includes, *inter alia*, a nucleic acid encoding an enzyme that catalyzes biosynthesis of lycopene, β-carotene, astaxanthin, or one of their precursors. All of the recited compounds are isoprenoids. The amendment to claim 12 finds support in the specification, which teaches enzymes for the biosynthesis of isoprenoids (e.g., page 4, lines 6-18).

Claim 21 has been amended to depend from claim 13, which is currently amended to be an independent claim. Amended claim 13 covers an *E. coli* host cell that includes, *inter alia*, a nucleic acid sequence encoding isopentenyl diphosphate isomerase, geranylgeranyldiphosphate synthase, 1-deoxyxylulose 5-phosphate synthase, a phytoene synthase, or a phytoene desaturase. Support for this amendment appears in the specification, which teaches that these enzymes are required for the biosynthesis of isoprenoids, e.g., page 5, lines 1-22.

Accordingly, Applicants submit that amended claims 5, 21, 24, 40, and 45 are commensurate in scope with the enablement provided in the specification.

Withdrawal of the rejections is respectfully requested.

Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 13 and 41 were rejected as allegedly indefinite. Applicants have cancelled claim 41.

Regarding claim 13, the Office Action remarks that "[c]laim 13 is confusing in the recitation of the 'host cell of claim 17 wherein the enzyme is ... 1-deoxyxylulose 5-phosphate

synthase' because claim 17, from which claim 13 depends, limits the enzyme to an enzyme that catalyzes biosynthesis of an isoprenoid but the product of 1-deoxyxylulose 5-phosphate synthase (i.e., 1-deoxyxylulose 5-phosphate) is not an isoprenoid.

As mentioned above, claim 13 has been amended to be an independent claim that covers an *E. coli* host cell containing a nucleic acid expression cassette encoding any one of isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, 1-deoxyxylulose 5-phosphate synthase, a phytoene synthase, or a phytoene desaturase. Applicants submit that since amended claim 13 does not require direct biosynthesis of an isoprenoid (i.e., as a direct product of the activity of one of the enzymes), the above-cited ground for rejection of claim 13 is mooted.

Rejections under 35 U.S.C. § 103

Claims 1, 5, 40, and 45 were rejected as allegedly obvious over either of Khosla *et al.* (US PG-PUBS 2002/0045220; "Khosla") or in view of Bock *et al.* ("Bock"), McCleary *et al.* (reference AL of Applicant's PTO-1449; "McCleary AL"), McCleary *et al.* (reference AM of Applicant's PTO-1449; "McCleary AM") and Haldiman *et al.* ("Haldiman") or Feng *et al.* ("Feng"). The Office Action notes that "claims 12 and 55 are objected to as being dependent on a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims" (page 10, lines 17-20). The rejection of claim 1 is mooted as this claim was cancelled.

Claim 5 has been amended to depend from claim 12. As noted above, amended claim 12, now an independent claim, is not obvious in light of the Office Action's statement in the just-quoted passage and the fact that it includes the limitations of the base claim and the intervening claims.

Independent claim 40, covering an *E. coli* bacterial host cell, has been amended to recite, *inter alia*, a nucleic acid that includes a coding sequence encoding a specific biosynthetic enzyme, i.e., isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, 1-deoxyxylulose 5-phosphate synthase, phosphoenolpyruvate synthase, 3-ketoacyl reductase, poly-3 hydroxyalkanoate polymerase, a phytoene synthase, a phytoene saturase, lycopene cyclase, farnesyl diphosphate synthase, and an operably linked promoter that is bound by ntrC

and regulated by acetyl phosphate. The specification teaches that these enzymes are useful for biosynthesis of an isoprenoid or a polyhydroxyalkonate, e.g., page 5, lines 15-22.

After a discussion of each of the references, the Office Action remarks:

> [I]t would have been obvious to one of ordinary skill in the art to replace the [inducible] lac promoters in the constructs of [Khosla] with a promoter which is induced by high acetyl phosphate levels. As [McCleary AL and McCleary AM] teach that acetyl-phosphate levels correlate with the amount of acetyl-CoA produced, it would have been obvious to one of ordinary skill in the art to link the polyketide synthase genes of [Khosla] to the acetyl-phosphate regulated promoters taught by [Haldiman] or [Feng] and express these constructs in *E. coli* cells which lack the cognate histidine kinases such that the response regulators which activate transcription from these promoters are activated by acetyl phosphate. Furthermore, it would have been obvious to one of ordinary skill in the art to put the cells and vectors necessary for production of high levels of polyketides together in a kit for easy handling (Office Action, page 10, lines 2-16).

Applicants disagree. It is respectfully submitted that there is no motivation to combine the cited references, since, prior to this invention, acetate was generally considered detrimental to growth of bacterial cells and production of recombinant proteins. For example, Aristidou (Item BE in supplemental IDS filed May 16, 2003) states at column 1, page 475:

> Acetate is a lipophilic agent that is harmful to cell growth. Moreover, experimental results in our laboratory agree well with common observation that recombinant gene expression is greatly reduced for acetate accumulation above 15-25 mM.

Bauer (Item BE in supplemental IDS filed May 16, 2003) is to similar effect. See, for example, column 1, page 1296:

> Organic acids accumulate in the culture medium during aerobic growth of *Escherichia coli* on glucose. The most abundant organic acid is often acetic, and its concentrations can build up to levels that are inhibitory to growth. In a previous study, we showed that intracellular accumulation of interleukin-2 (IL-2) . . . was inversely correlated with cell density and acetate accumulation in fermentor cultures. These observations provided circumstantial evidence that acetate was at least partially responsible for the cessation of product accumulation during expression of heterologous genes in *E. coli* . . . .

Given these detrimental effects of acetate on recombinant protein production, one would not have been motivated to use a promoter that is regulated by acetyl phosphate to produce one

of the enzymes recited in amended claim 40. Applicant is unaware of any teaching in Khosla, Bock, McCleary AL, McCleary AM, Haldiman, and Feng to the contrary.

Prior to the Applicant's invention, high acetate levels might have been expected to impair production of a heterologous protein, such as phosphoenolpyruvate synthase recited in claim 40, to the extent that a sufficient amount of this enzyme would not be produced to counteract the accumulation of acetate. Thus, one would not have been motivated to combine Kholsa and Bock with McCleary AL, McCleary AM, Haldiman, and Feng. Only with the hindsight provided by this application can one conclude that a waste or starvation signal such as acetate should be used to induce expression of a heterologous polypeptide that catalyzes a reaction in a metabolic pathway that produces an isoprenoid.

For at least these reasons, Applicant respectfully submits that the rejection of claims 45 based on Kholsa in view of Bock, McCleary AL, McCleary AM, Haldiman, and Feng should be withdrawn.

CONCLUSION

Based on the remarks set forth above, Applicant submits that all of the pending and new claims cover allowable subject matter. Allowance by the Examiner, and rejoinder of method claims that refer to allowable host cells are respectfully solicited.

No fee is believed due. Please apply any other charges or credits to deposit account 06-1050 referencing attorney docket No. 06497-013002.

Respectfully submitted,

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